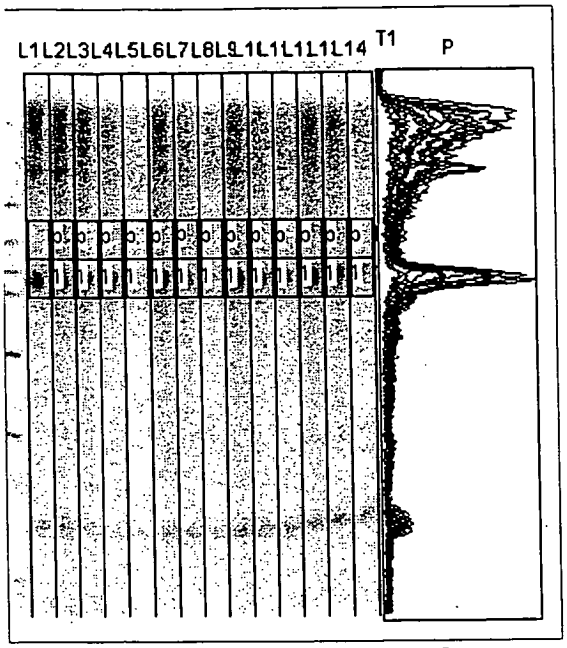
DOCUMENT I

INHIBITION OF HIV AND HSV TRANSCRIPTION BY METHYLATED NOGA

WORK WAS ACCOMPLISHED IN THE FIRST 6 MONTHS OF 1996 IN ROOM 249 LEVI BUILDING, JOHNS HOPKINS UNIVERSITY, INUENTORS LAB.

File:012696.IMG Acquired:1/29/96 11
huang
Bitmap Display Range:0-63 counts, li

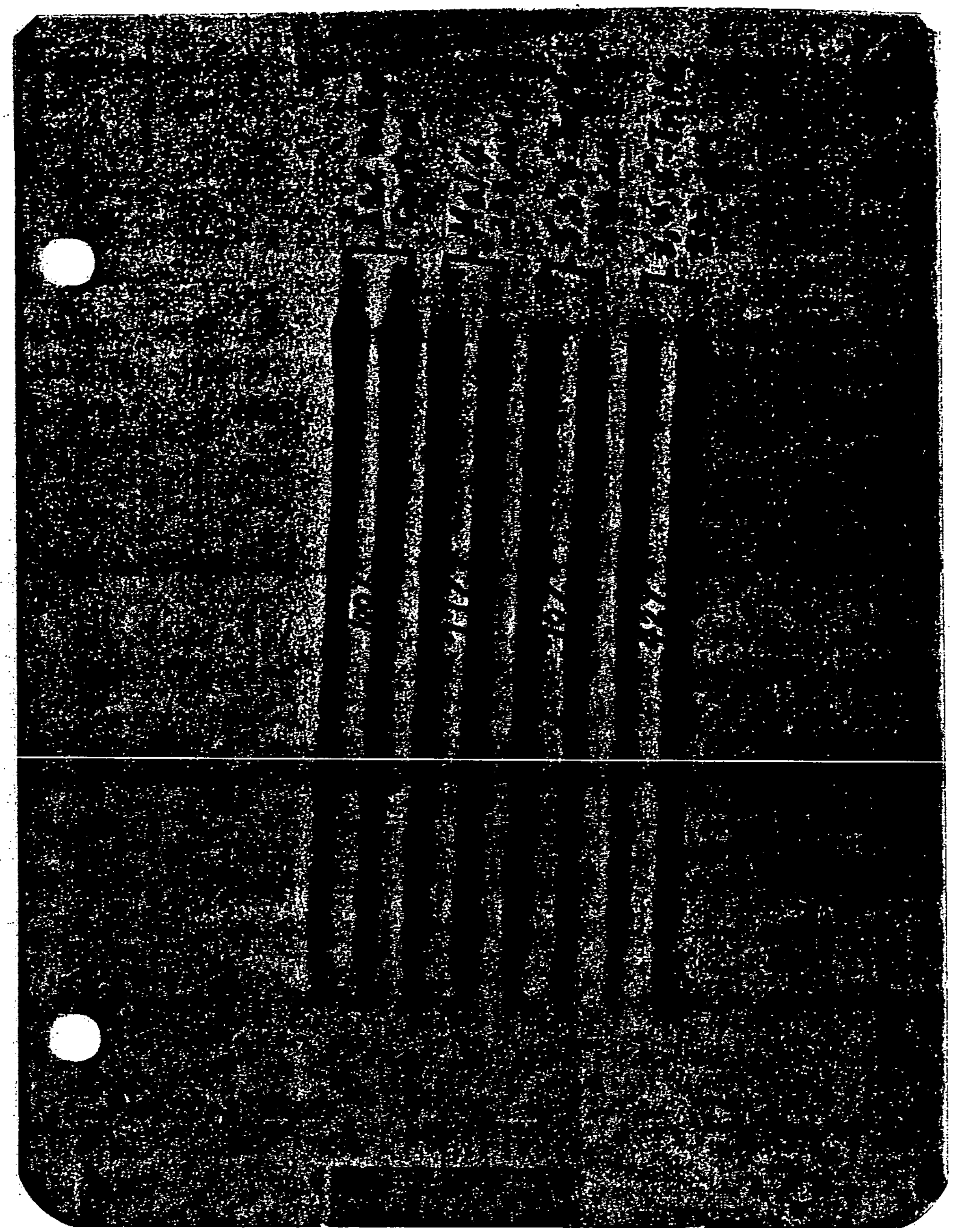
#T1 Lanes
Background Subtraction: regions



ID	Dist (mm)	Type	Gross Counts			
Lane #1	**Bkgd subtr =12.62/sqmm**					
2	42.5	Bkgd	757			
1	53.0	Unkn	3,631			
Lane #2	**Bkgd subtr =19.38/sqmm**					
2	42.5	Bkgd	1,163			
1	53.0	Unkn	3,464			
Lane #3	**Bkgd subtr =16.07/sqmm**					
2	42.5	Bkgd	964			
1	53.0	Unkn	2,832			
Lane #4	**Bkgd subtr =12.20/sqmm**					
2	42.5	Bkgd	732			
1	53.0	Unkn	1,936			
Lane #5	**Bkgd subtr =6.13/sqmm**					
2	42.5	Bkgd	368			
1	53.0	Unkn	635	368	267	
Lane #6	**Bkgd subtr =17.23/sqmm**					
2	42.0	Bkgd	1,034			
1	53.0	Unkn	3,575	1,034	2,541	
Lane #7	**Bkgd subtr =11.30/sqmm**					
2	42.5	Bkgd	678			
1	53.0	Unkn	1,764	678	1,086	
Lane #8	**Bkgd subtr =8.08/sqmm**					
2	42.5	Bkgd	485			
1	53.0	Unkn	821	485	336	
Lane #9	**Bkgd subtr =16.23/sqmm**					
2	42.5	Bkgd	974			
1	53.0	Unkn	3,012	974	2,038	
Lane #10	**Bkgd subtr =10.90/sqmm**					
2	42.5	Bkgd	654			
1	53.0	Unkn	1,680	654	1,026	
Lane #11	**Bkgd subtr =8.38/sqmm**					
2	42.5	Bkgd	503			
1	52.5	Unkn	1,241	503	738	

ID	Dist (mm)	Type	Gross Counts	Bkgd Subtr	Net Total
Lane #1	**Bkgd subtr =12.62/sqmm**				
2	42.5	Bkgd	757		
1	53.0	Unkn	3,631	757	2,874
Lane #2	**Bkgd subtr =19.38/sqmm**				
2	42.5	Bkgd	1,163		
1	53.0	Unkn	3,464	1,163	2,301
Lane #3	**Bkgd subtr =16.07/sqmm**				
2	42.5	Bkgd	964		
1	53.0	Unkn	2,832	964	1,868
Lane #4	**Bkgd subtr =12.20/sqmm**				
2	42.5	Bkgd	732		
1	53.0	Unkn	1,936	732	1,204
Lane #5	**Bkgd subtr =6.13/sqmm**				
2	42.5	Bkgd	368		
1	53.0	Unkn	635	368	267
Lane #6	**Bkgd subtr =17.23/sqmm**				
2	42.0	Bkgd	1,034		
1	53.0	Unkn	3,575	1,034	2,541
Lane #7	**Bkgd subtr =11.30/sqmm**				
2	42.5	Bkgd	678		
1	53.0	Unkn	1,764	678	1,086
Lane #8	**Bkgd subtr =8.08/sqmm**				
2	42.5	Bkgd	485		
1	53.0	Unkn	821	485	336
Lane #9	**Bkgd subtr =16.23/sqmm**				
2	42.5	Bkgd	974		
1	53.0	Unkn	3,012	974	2,038
Lane #10	**Bkgd subtr =10.90/sqmm**				
2	42.5	Bkgd	654		
1	53.0	Unkn	1,680	654	1,026
Lane #11	**Bkgd subtr =8.38/sqmm**				
2	42.5	Bkgd	503		
1	52.5	Unkn	1,241	503	738

Lane #12		**Bkgd subtr =15.18/sqmm**			
2	42.5	Bkgd	911		
1	52.5	Unkn	3,114	911	2,203
Lane #13		**Bkgd subtr =11.92/sqmm**			
2	42.5	Bkgd	715		
1	52.5	Unkn	2,438	715	1,723
Lane #14		**Bkgd subtr =6.07/sqmm**			
2	42.5	Bkgd	364		
1	52.0	Unkn	930	364	566

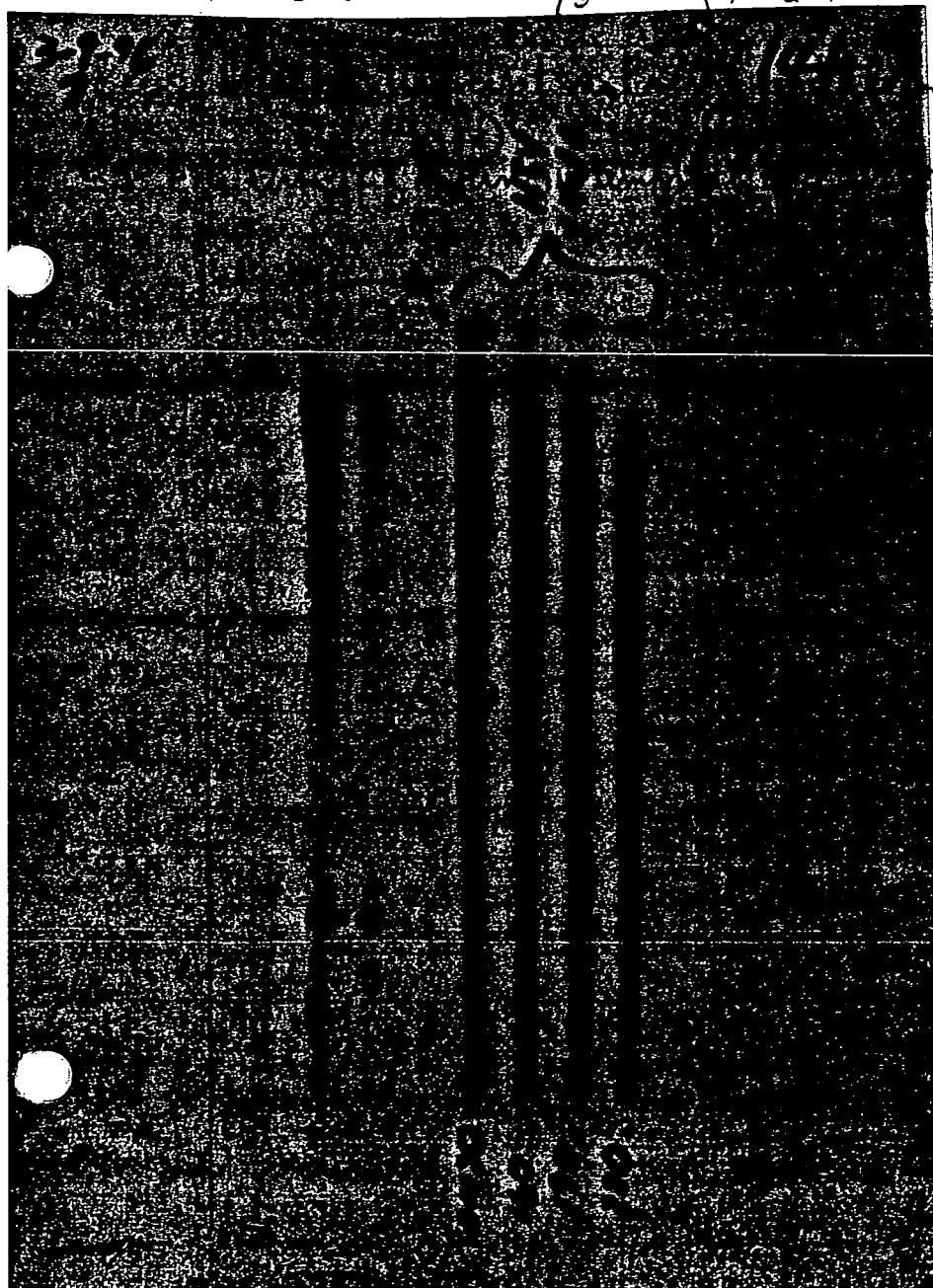


2-29-96 Test of HSV - pBR 322 Δ 380 template inhibition by Mal 4

Will use 300 nanograms template in 12.8 λ. Will do Marley at 60% conc. at 160, 190 and 220 ng/rxn Mal 4.

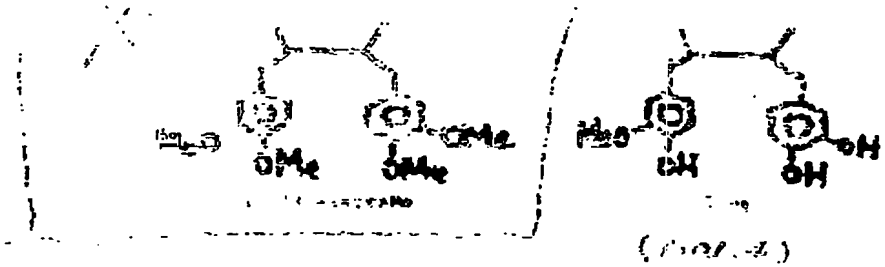
1. DMSO cntrl .4 λ
2. Mal 4 160
3. 190
4. 220

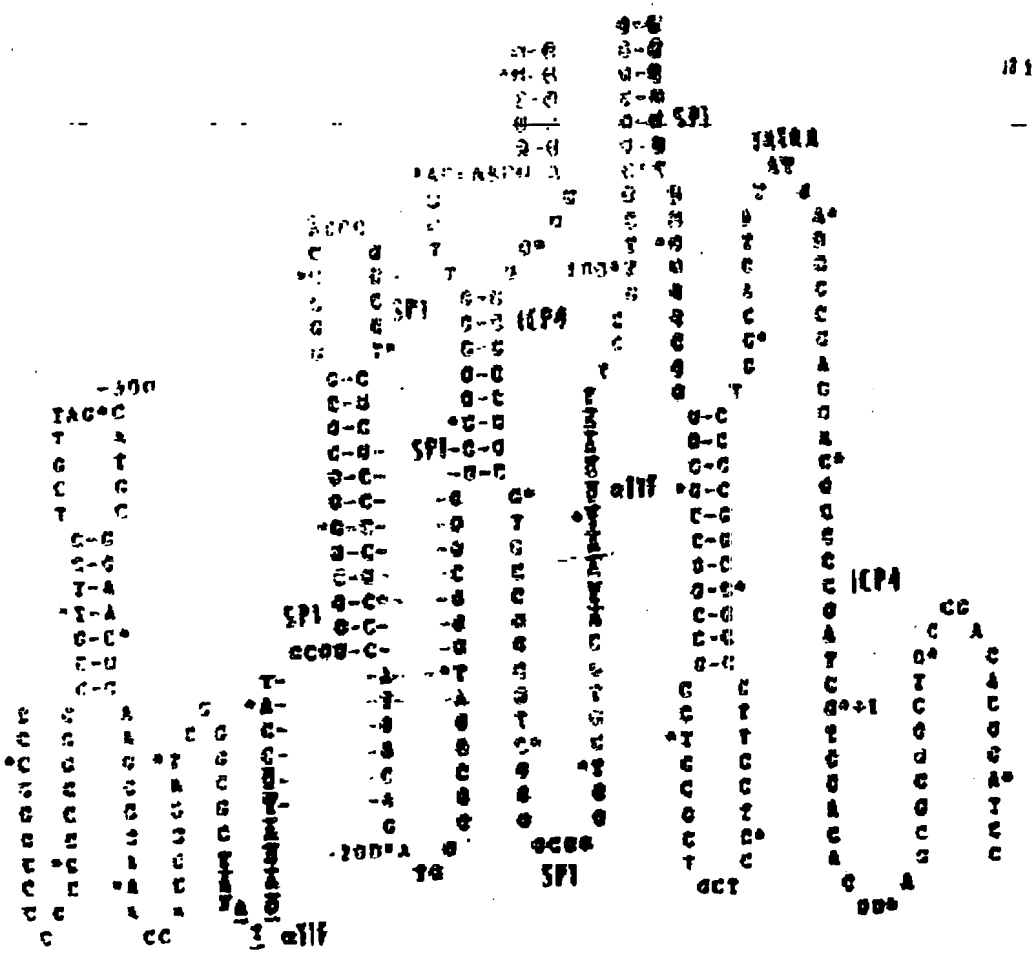
Dissolve DNA in 9.6 λ H_2O. Add. 4 λ Mal 4 dilutions and incubate 15 min at 30°. For 60% Marley need 4.5 λ extract + 3 λ buffer.



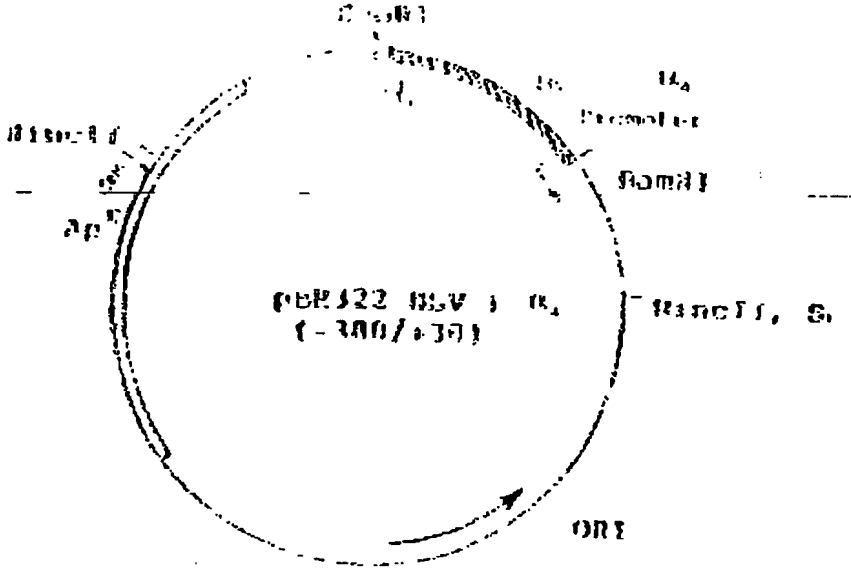
t using 50% extract. Loaded 1/2 Rx.

t the 160, 190, 220 Mal 4.





B Template of HSV I α_4



C. Effect of Mal 4 on the promoter activity of α_4 gene *in vitro*

Template	% Transcription (Mal 4 µg/ml)				
	0	100	130	160	190
pBR322 HSV I α_4	100	20	0	6	0

[Ref 32. Mackem, S. & Roizman, B.

DOCUMENT II

A. DESIGN AND INSTRUCTION FROM DR. RUCHIH C. HUANG, INVENTOR, TO DR. H. S. CHEN FOR CARRYING OUT EXPERIMENTS USING TETRAMETHYL NDGA TO INHIBIT HSV IN VERO CELLS AND TOXICITY STUDIES OF THE DRUG IN MICE

LETTER DATED IN MAY 21 AND MAY 24, 1996

B. RESULTS RECEIVED BY DR. HUANG 6/25/96

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410 516-7330 FAX (410) 516 5213

DR. CHEN HONGSHAN
Fax 516-746-6618

NDGA 2mg/50ml DMSO. 于2005-7-3

鴻翔教授:

① NDGA 与 MA1 4瓶, 今日 FED EXPRESS 寄上 TETRAMETHYL

② 体外抑制 HIV integrase 的工作, NDGA 250ug 在一瓶 DMSO [illegible]

③ NDGA 对 HSV-1 之 4 个细胞的效果, 每只, [illegible] Kg

T[illegible] NIH IA [redacted] of HSV-1 動物實驗 [illegible]

DOSE: 2mg/50ml ([illegible]) 每日一次, 共注射 5-7 天.

注射方式 可以是 IV, IP [illegible]

至於 HSV-1 [illegible] 已決定, 又由 [illegible] 定. TETRAMETHYL NDGA 在 [illegible] (Sp1) 之 TRANSCRIPTION 之一為 抑制 HSV-1 REPLICATION [illegible] 等於 [illegible] 之 [illegible] 解釋 (見 [illegible] PROMOTER 部位之 Sp1 sites).

JOHNS HOPKINS
UNIVERSITY

Department of Biology
[illegible] Hall [illegible] Charles Street
Baltimore MD 21218-2685
(410) 516-[illegible] / FAX (410) 516-[illegible]

1996年5月24日

回傳一次。

DR. CHEN HONGSHAN
Fax 516-746-6615

[illegible]

*

今日 FED EXPRESS 寄上 TETRA METHYL NDGA 250mg 及一瓶 DMSO 寄上，作 INHIBITION OF HSV-1 動物實驗，老鼠 (MOUSE)

DOSE: 2mg/50ul (50 ul INOCULATION);
每日一次，共連續 [illegible] 天。

注射方式 [illegible]。

至於 HSV-1 infection 方式 [illegible] 自己決定，又如何查看效果亦由你決定。TETRA METHYL NDGA 在 [illegible] (Sp_1) 之 TRANSCRIPTION 是一種 [illegible] HSV-1 REPLICATION [illegible] 學術 [illegible] (見 [illegible] PROMOTER 部份之 SP1 [illegible])

* 要直接溶在 100% DMSO 中 每天/每次 打 0.05ml 含 TETRA-methyl NDGA 2mg

II B

1996.6.25 給葉開女士

C-4N DOC

Table 1. Cytotoxicity and inhibition of HSV-1 CPE by 4N in vero cell cultures

1996, 6-20-25

Agent	Conc. ug/ml	Cytotoxicity(OD570) X ± SD	Inhibition of cell growth %	TC50 ug/ml	HSV-1 CPE (OD570) X ± SD	Inhibition of HSV-1CPE %	IC50 ug/ml
4-N	1000.00	0.03, 0.03, 0.03, 0.02 0.03 ± 0.01	97.05	83	0.04, 0.05, 0.00, 0.00 0.02± 0.03	-	<0.98
	500.00	0.03, 0.04, 0.05, 0.04 0.04± 0.01	96.08		0.05, 0.11, 0.07, 0.04 0.07± 0.03	-	
	250.00	0.30, 0.30, 0.30, 0.30 0.3± 0	70.59		0.30, 0.26, 0.22, 0.28 0.27± 0.03	-	
	125.00	0.34, 0.38, 036, 0.39 0.37± 0.02	63.73		0.57, 0.45, 0.62, 0.60 0.56± 0.08	31.5	
	62.50	0.45, 0.46, 0.45, 0.45 0.45± 0.01	55.88		0.52, 0.49, 0.68, 0.66 0.59± 0.10	34.8	
	31.30	0.81, 0.80, 0.86, 0.85 0.83± 0.03	18.63		0.90, 0.84, 0.79, 0.87 0.85± 0.05	64	
	15.60	1.08, 1.02, 1.00, 0.98 1.02± 0.04	0		0.72, 0.92, 0.68, 0.92 0.81± 0.13	59.6	
	7.80	1.25, 1.04, 1.00, 1.11 1.10± 0.11	-0.08		0.74, 0.79, 0.96, 1.03 0.88± 0.14	67.4	
	3.90	1.10, 1.08, 1.06, 1.08 1.08± 0.02	-0.06		0.79, 1.07, 0.83, 0.76 0.86± 0.14	65.2	
	1.95	1.10, 1.20, 1.22, 1.00 1.10± 0.1	-0.08		0.78, 0.85, 0.81, 0.76 0.80± 0.04	58.4	
	0.98	1.30, 1.25, 1.15, 1.15 1.20± 0.08	-0.18		0.83, 0.73, 0.80, 0.83 0.80± 0.05	58.4	
	CC	1.02, 1.03, 1.00, 1.04 1.02± 0.02			1.13, 1.22, 1.18, 1.15 1.17± 0.04		
	vc X± SD				0.24, 0.29, 0.31, 0.26 0.28± 0.03		

Table 2. Inhibition of HSV-1 CPE by ACV on vero cell

1996, 6, 20-25.

Agent	conc. ug/ml	HSV-1 CPE(OD570)	Inhibition %	IC50 ug/ml
ACV	1000	0.70,0.53,0.57,0.62	37	<0.98
	X± SD	0.61± 0.07		
	500	0.63,0.38,0.62,0.49	28.1	
	X± SD	0.53± 0.12		
	250	0.50,0.49,0.64,0.87	39.3	
	X± SD	0.63± 0.18		
	125	0.41,0.43,0.53,0.66	25.8	
	X± SD	0.51± 0.11		
	62.5	0.73,0.72,0.73,0.76	51.7	
	X± SD	0.74± 0.02		
	31.3	0.93,0.90,0.86,0.93	70.8	
	X± SD	0.91± 0.03		
	15.6	1.00,0.99,0.98,1.02	80.9	
	X± SD	1.00± 0.02		
	7.8	1.09,1.11,1.16,1.10	94.4	
	X± SD	1.12± 0.03		
	3.9	1.13,1.07,1.10,1.09	92.1	
	X± SD	1.10± 0.03		
	1.95	1.14,1.12,1.17,1.11	96.6	
	X± SD	1.14± 0.03		
	0.98	0.76,0.80,0.82,0.92	61.8	
	X± SD	0.83± 0.07		
	CC	1.13,1.22,1.18,1.15		
	X± SD	1.17± 0.04		
	VC	0.24,0.29,0.31,0.26		
	X± SD	0.28± 0.03		

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医药生物技术研究所

4N 与 Vero 细胞毒性　　96. 6. 25

药量 μg/ml	细胞 OD 值 (570)	破坏%	IC_{50}
1000	0.03 0.03 0.03 0.02	97.05	83 μg/ml
$\bar{X} \pm SD$	0.03 ± 0.01		
500	0.03 0.04 0.05 0.04	96.08	
	0.04 ± 0.01		
250	0.3 0.3 0.3 0.3	70.59	
	0.3 ± 0		
125	0.34 0.38 0.36 0.39	63.73	
	0.37 ± 0.02		
62.5	0.45 0.46 0.45 0.45	55.88	
	0.45 ± 0.01		
31.3	0.81 0.80 0.86 0.85	18.63	
	0.83 ± 0.03		
15.6	1.08 1.02 1.00 0.98	0	CC 1.02 1.03 1.00
	1.02 ± 0.04		1.02 ± 0.02
7.8	1.25 1.04 1.00 1.11	-0.08	
	1.1 ± 0.11		VC 0 0 0.0
3.9	1.10 1.08 1.06 1.08	-0.06	
	1.08 ± 0.02		
1.95	1.10 1.20 1.22 1.00	-0.08	
	1.1 ± 0.1		
0.98	1.30 1.25 1.15 1.15	-0.18	
L605	1.2 ± 0.08		

②

医药生物技术研究所

4N HSV-I 抑制实验 9.6.6.才

药品 μg/ml	OD值 (570 nm)	抑制率 %	IC_{50} (μg/ml)
1000	0.04 0.05 0.00 0.00	92.86	< 0.98
	0.02 ± 0.03		
500	0.05 0.11 0.07 0.04	75.50	
	0.07 ± 0.03		
250	0.30 0.36 0.20 0.28	3.57	
	0.27 ± 0.03		
125	0.53 0.45 0.62 0.60	31.5	
	0.56 ± 0.08		
62.5	0.52 0.49 0.68 0.66	34.8	
	0.59 ± 0.10		
31.3	0.90 0.84 0.79 0.87	64.00	
	0.85 ± 0.05		
15.6	0.75 0.92 0.68 0.92	59.6	
	0.81 ± 0.13		
7.8	0.74 0.79 0.96 1.03	67.4	
	0.88 ± 0.14		
3.9	0.79 1.07 0.83 0.76	62.2	
	0.86 ± 0.14		
1.95	0.78 0.85 0.81 0.76	58.4	
	0.80 ± 0.04		
0.98	0.83 0.73 0.80 0.83	58.4	
	0.80 ± 0.05		
CC	1.13 1.22 1.18 1.15		
	1.17 ± 0.04		
VC L605	0.24 0.29 0.31 0.26		
	0.28 ± 0.03		

(3)

医药生物技术研究所

ACN对HSV-I作用 96.6.5

药液 μg/ml	OD值	抑制率%	IC50 μg/ml
1000	0.70 0.53 0.57 0.62	37	<0.98
	0.61±0.07		
500	0.63 0.38 0.62 0.49	>85.1	
	0.53±0.12		
250	0.50 0.49 0.64 0.87	39.3	
	0.63±0.8		
125	0.41 0.43 0.53 0.66	>5.8	
	0.51±0.11		
62.5	0.73 0.73 0.73 0.76	51.)	
	0.74±0.02		
31.3	0.93 0.90 0.86 0.93	70.8	
	0.91±0.03		
15.6	1.00 0.99 0.98 1.02	80.9	
	1.00±0.02		
7.8	1.09 1.11 1.16 1.10	94.4	
	1.12±0.03		
3.9	1.13 1.07 1.10 1.09	92.1	
	1.10±0.03		
1.95	1.14 1.12 1.17 1.11	96.6	
	1.14±0.03		
0.98	0.76 0.80 0.82 0.92	61.8	
	0.83±0.07		
CC	1.13 1.22 1.18 1.15		
	1.17±0.04		
VCL605	0.24 0.29 0.31 0.26		
	0.28±0.03		

实验者：陈鸿珊 [illegible]

LOAL 6.20